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\TENT COOPERATION TRE Y

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
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United States Patent and Trademark Office, PCT
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Arlington, VA 22202
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in its capacity as elected Office

Date of mailing (day/month/year) 27 February 2001 (27.02.01)	
International application No. PCT/SG00/00077	**Applicant's or agent's file reference** 1161.P004PCT
International filing date (day/month/year) 01 June 2000 (01.06.00)	**Priority date** (day/month/year) 04 June 1999 (04.06.99)
Applicant SAM, Fong, Yau, Li et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

04 January 2001 (04.01.01)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

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PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
1161.P004PCT		
International application No.	International filing date (*day/month/year*)	Priority Date (*day/month/year*)
PCT/SG 00/00077	1 June 2000 (01.06.2000)	4 June 1999 (04.06.1999)

International Patent Classification (IPC) or national classification and IPC

IPC^7: G01N 27/447

Applicant

CE Resources PTE LTD. et al.

1. This international preliminary examination report has been prepared by this International Preliminary Examination Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 4 sheets, including this cover sheet.

☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of _____ sheets.

3. This report contains indications relating to the following items:

- I. ☒ Basis of the opinion
- II. ☐ Priority
- III. ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV. ☐ Lack of unity of invention
- V. ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI. ☐ Certain documents cited
- VII. ☐ Certain defects in the international application
- VIII. ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
4 January 2001 (04.01.2001)	20 July 2001 (20.07.2001)
Name and mailing address of the IPEA/AT Austrian Patent Office Kohlmarkt 8-10 A-1014 Vienna Facsimile No. 1/53424/200	Authorized officer NARDAI Telephone No. 1/53424/347

Form PCT/IPEA/409 (cover sheet) (July 1998)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/SG 00/00077

I. Basis of the report

1. With regard to the **elements** of the international application:*

☒ the international application as originally filed

☐ the description:
pages _____ . as originally filed
pages _____ . filed with the demand
pages _____. filed with the letter of _____.

☐ the claims:
pages _____ . as originally filed
pages _____ . as amended (together with any statement) under Article 19
pages _____ . filed with the demand
pages _____. filed with the letter of _____.

☐ the drawings:
pages _____ . as originally filed
pages _____ . filed with the demand
pages _____ . filed with the letter of _____.

☐ the sequence listing part of the description:
pages _____ . as originally filed
pages _____ . filed with the demand
pages _____ . filed with the letter of _____.

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is:

☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

☐ the language of publication of the international application (under Rule 48.3(b)).

☐ the language of the translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

☐ contained in the international application in printed form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. ☐ The amendments have resulted in the cancellation of:

☐ the description, pages _____.

☐ the claims, Nos. _____.

☐ the drawings, sheets/fig _____.

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

** Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

*** Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.*

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/SG 00/00077

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1-27	YES
	Claims		NO
Inventive step (IS)	Claims	1-27	YES
	Claims		NO
Industrial applicability (IA)	Claims	1-27	YES
	Claims		NO

Citations and explanations (Rule 70.7)

JP 11 108890 A (abstract) shows a detecting element for an electric potential gradient detection type electrophoresis apparatus comprising a pair of electric potential gradient detection type electrodes, which are mounted parallel to a glass board. A part of the electrodes are exposed to the flow path.
The above cited document does not disclose (according to the independent claims 1,2 and 3 respectively)

- an electrophoretic apparatus comprising:
a power supply with a ground electrode and a power electrode;
a separation channel with an inlet end and an outlet end and containing separation medium, said inlet end electrically coupled to the power electrode of said power supply, said outlet end electrically coupled to the ground electrode of said power supply;
a data acquisition system with a reference electrode and an electrical potential sensing electrode, said reference electrode electrically coupled to said ground electrode; and
a conductive element, provided on said separation channel between said inlet end and said outlet end, said conductive element permitting electrical signals to pass through without detactable bulk flow of separation medium and sample, said sensing electrode electrically coupled to said conductive element.

- an electrophoretic apparatus comprising:
a capillary electrophoresis array chip containing
a plurality of longitudinally aligned capillary separation channels containing separation medium, each said channel having an inlet end and an outlet end,
a conductive element provided on each of said separation channel proximate the corresponding outlet end, said conductive element allowing electrical signals to pass through without detectable bulk flow of the separation medium and sample;
a plurality of sensor reservoirs containing electrically conductive medium each connected to one of said conductive element of said separation channels.

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: Box V (page 1)

- a method for detecting samples in an electrophoretic system, said system containing an electrophoretic channel with an inlet end and an outlet end, said channel containing electrophoretic medium and samples, said apparatus further containing an electrically conductive element on the wall of said electrophoretic channel proximate said outlet end, said conductive element permitting electrical signal to pass through without detectable bulk flow of electrophoretic medium and sample.

The dependent claims 4 to 27 show further embodiments.

Industrial applicability is obviously given.

POTENTIAL GRADIENT DETECTOR FOR ELECTROPHORESIS

FIELD OF THE INVENTION

The present invention is related to sample detection in electrophoresis. In particular, the present invention is related to conductivity detection in electrophoresis.

BACKGROUND OF THE INVENTION

Capillary electrophoresis (CE) is a powerful analytical separation technique for the analysis of complex mixtures. In CE, an unknown sample is introduced at an inlet of a capillary channel filled with a buffer solution, and a high voltage is applied across a section of the capillary. Different constituents of the sample migrate through the capillary at different rates depending on their electrophoretic mobility's, and are separated into different zones. By detecting the chemicals passing through a part of the capillary or its outlet as a function of time, and knowing the of the possible constituents, the chemical composition of the sample can be determined. A number of detectors have been developed for CE, including optical and electrochemical methods. Electrochemical detection can be classified into three main categories: amperometry, voltammetry and conductivity measurements. Conductivity detection is a non-selective detection mode and universally applicable. Analytes are detected because of their different conductivities to that of the background electrolyte.

One method to measure conductivity during electrophoresis is potential gradient detection, which is accomplished by putting two electrodes in the applied electric field for electrophoresis and detecting sample components by measuring potential changes between these two electrodes when sample components are passing by. This method has been used for isotachophoresis (US3941678, 20 Feb. 1975 and US3932264, 13 Jan. 1976) and it has been mentioned that such a method can be used in modern CE (F. Foret, L. Krivankova and P. Bocek, Capillary Zone Electrophoresis, chapter 7, p147-150). There are, however, problems for using this method in electrophoresis. Firstly, the sensing electrodes need to be inserted into the separation column or capillary. The procedures are troublesome and tedious, especially if the inner diameter of the capillary is small, for example in the case of capillary electrophoresis (usually between 10 –100 μm). The more serious problem is that the sensing electrodes are polarized during electrophoresis. In order to prevent formation of bubbles and deposits on the electrodes so that the electrophoresis processes can be performed under stable conditions and high sensitivity can be obtained, special means have to be used, such as adopting v/F and F/v converters in the instrumental design, reducing the areas of electrodes contacting with the buffer solutions and adding nonionic surfactant. However, all these means can only serve to alleviate, but can't eliminate completely the problems encountered.

Therefore, conductivity detection is usually accomplished by measuring the potential difference (signal) between two electrodes while passing through a small constant current (excited source). Several designs are

used for conductivity det ction in CE, i.e., on-column, end-column and contactless structures. On-column detection cells (Anal. Chem., 1987, 59, 2747-2749, US Patent No. 5223114, 29 June 1993 and US Patent, No 5580435, 1994) are usually made by inserting two sensing platinum wires into the separation capillary so that the sensing electrodes can directly contact the electrolyte solution in the capillary. Although on-column conductometric detection works well, the question arises as to how to produce such structures reliably and inexpensively. The more common practices are the use of end-column detectors (such as those disclosed in Anal. Chem. 1991, 63, 189-192, J. of Capillary Electrophoresis, 1996, 1:1-11, US patent 5298139, and US patent 5126023), which have the advantage that the sensing electrode can be constructed directly at the outlet of the separation capillary. For end-column detection, the correct alignment of the sensing electrode with the outlet of the separation capillary is critical for success. However, the alignment is usually difficult due to the small inner diameter (10 μm –100μm) of the capillary.

Another solution offered in the prior art is contactless conductivity detection (Anal. Chem., 1998, 70, 563-567). In this method, two electrodes are laid on the outside wall of the separation capillary. Therefore, no electrode is in contact with electrolyte solution. However, it is generally accepted that the contactless detection is not sensitive enough. Although their structures are varied, all the prior designs should use their own excited source and considered the high voltage applied for electrophoresis as a noise source.

Although the three techniques described above (i.e. potential gradi nt detection, potential difference detection, and contactless conductivity detection) are all based on the difference in conductivity between the electrophoretic medium and the samples, potential difference detection is the most widely used technique in capillary electrophoresis. Therefore, commercially available and commonly described conductivity detection systems typically employ the potential difference detection method.

OBJECT OF THE INVENTION

It is therefore an object of the present invention to provide a conductivity detection system which obviates the need to insert electrodes into the separation channel.

It is another object to provide a conductivity detection system which is effective and sensitive.

SUMMARY OF THE INVENTION

The present invention provides an on-column electrochemical detector based on the principle of potential gradient detection for electrophoresis of samples in which the electrodes for detection are physically isolated from the electrophoretic separation channel, but maintain the same electrical potential as the corresponding interior of the electrophoretic separation channel. Since the sensing electrodes are not in direct contact with the electrophoretic medium within the electrophoretic channel, problems due to bubble and deposit formation are eliminated.

The apparatus can make use of conventional separation channel with an inlet end connected to an inlet reservoir and an outlet end connected to an outlet reservoir. In accordance with the present invention, a sensor reservoir with electrically conductive medium is added and connected to the separation channel via a conductive element on the surface of the separation channel. A sensing electrode is submerged in the electrically conductive medium within the sensing reservoir. The conductive element allows electrical potential from the interior of the separation channel to be

transferred to the sensor reservoir without detectable bulk flow of electrophoretic medium or samples. Detectable bulk flow refers to the movement of solute or sample to an extent that there is detectable interference or disruption to migration of the sample. This detection may be based standard detection methods or the method described in the present invention. In one embodiment, the conductive element is a fracture in a separation channel made of fused silica tubing. In another embodiment, the conductive element is a thin layer of porous glass on the wall of a capillary channel electrophoretic chip.

During electrophoresis, the channel and reservoirs are filled with electrophoretic medium, and the ground and power electrodes from a power supply are connected to the outlet and inlet reservoirs respectively. Sample detection is achieved by sensing the potential gradient between the conductive element and the outlet end where the sensing and reference electrodes are respectively connected in the preferred embodiment. The distance between the element and the outlet end is also preferably as small as the length of the sample plug in order to maximize sensitivity of detection and resolution.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1A and B are typical detection methods described in the prior art.

Fig. 2A is a schematic diagram of a capillary electrophoresis system as described in one embodiment of the present invention.

Fig. 2B is an enlarged view of area G as shown in Fig. 2A.

Fig. 3A shows the electric field as detected by the data acquisition system using the capillary electrophoretic system shown in Fig.2A without sample.

Fig.3B shows the electric field as detected by the data acquisition system using the capillary electrophoretic system shown in Fig.2A when sample is injected and separated into zones.

Fig. 4 is a schematic diagram of the microchip CE system to illustrate another embodiment of the invention.

Fig. 5 is an enlarged view of area H as shown in Fig.4.

Fig. 6 is a block diagram of a circuit for the detector to illustrate yet another embodiment of the present invention.

Fig. 7 is an electropherogram obtained using the system shown in Fig. 2A.

Fig. 8 is an electropherogram obtained on microchip CE using the system shown in Fig. 4.

Fig. 9 is a schematic diagram of yet another embodiment of the present invention.

DESCRIPTION OF THE INVENTION

The following detailed description describes the preferred embodiment for implementing the underlying principles of the present invention. One skilled in the art should understand, however, that the following description is meant to be illustrative of the present invention, and should not be construed as limiting the principles discussed herein. In addition, certain terms are used throughout the following description and claims to refer to particular system components. As one skilled in the art will appreciate, companies may refer to a component by different names. This document does not intend to distinguish between components that differ in name but not in function. For example, the pair of electrodes for electrophoresis are referred to herein as "ground" and "power" electrodes for clarity of description. It is understood by one skilled in the art that the ground electrode may be at zero volts or floating, and that the power electrode may be of positive or negative polarity. For the same reason of clarity of description, the pair of electrodes for potential gradient detection are referred to herein as "sensing" and "reference" electrodes. It should be understood that the "sensing" electrode could be the same type as the "reference" electrode. Their positions can be exchanged with each other without affecting detection results. When performing electrophoresis on microchip, at least four electrodes are often needed for sample introduction and separation. For ease of understanding, these electrodes are classified as "power", "ground", "sample" and "waste" electrodes. It should be understood that the exact pot ntial on these electrodes are not fixed, and may be set up according

to the needs of the user. The reservoirs on the microchip have also been given the nam s "inlet", "outlet", "sample" and "waste" reservoirs for clarity of description. It should also be understood that the reservoirs can be used to contain different medium depending on the experimental conditions required. Furthermore, no particular inlet and outlet reservoir structures are required if microinstruments are used to load small quantities of samples directly into the inlet end.

In the following discussion, and in the claims the terms "including", "having" and "comprising" are used in an open-ended fashion, and thus should be interpreted to mean "including but not limited to .....". Also, the term " or "couples" is intended to mean either an indirect or direct electrical connection. Thus if a first device "couples" to a second devices, that connection may be a direct electrical connection or through an indirect electrical connection via other devices, electrical conductive medium or connections. Capillary electrophoresis is used for purposes of illustration. It should be understood by one skilled in the art that the same principles may be applied to other types of electrophoretic separations, using the teaching provided herewith.

Figures 1A and B show a section of an electrophoretic capillary tube, illustrating the principles of potential gradient and potential difference detection systems respectively as known in the art. In the potential gradient detection system as shown in Figure 1A, the two sensing electrodes **10A** and **10B** come into contact with the electrophoretic medium at two non-parallel positions along the longitudinal axis of the electrophoretic channel **12**, which is connected to a power source

generating an electrical potential between ends **12a** and **12b**. The portions of the electrodes in contact with the solution inside the capillary tube is shown in dotted lines. In the potential difference detection method, the two sensing electrodes **14A** and **14B** have to be in contact with the electrophoretic medium at exactly the same cross-sectional plane of channel **15** having an electrophoretic potential between ends **15a** and **15b**. The resistance of the electrophretic medium may be monitored using a small a.c. current between the sensing electrodes. Because the sensing electrodes are within high electric field during electrophoresis, bubble and deposit form on the surface of the sensing electrodes due to electrochemical reactions, which would affect the electrophoretic process, and decrease the detection sensitivity since the sensing electrodes are directly within the electrophoretic channel.

Figures 2A and 2B show one setup of capillary electrophoresis (CE) based on potential gradient detection constructed in accordance with the present invention. A 50 μm inner diameter fused silica capillary is used as the separation capillary **32**. A fracture **58** is made before the outlet of the separation capillary **32**. The distance L between the fracture **58** to the outlet, usually between 0.1 mm to 5 mm, should be near or smaller than the length of the sample plug injected into the separation capillary **32** in order to obtain maximum resolution between separated peaks. The capillary **32** is then inserted into the buffer reservoirs so that the outlet **37** of the capillary is connected to outlet reservoir **38**, fracture **58** is submerged in sensor reservoir **34**, and inlet **31** of the capillary is inserted into inlet reservoir **30**. Good insulation between reservoirs **34** and **38** is made by

using an insulation layer **54**. Running buffer solutions for electrophoresis are filled into the three buffer reservoirs as well as the bore of the separation capillary **32**. The ground and power electrodes **46** and **26** are connected with the high voltage power supply **20** to apply high voltages needed for electrophoresis. Sensing electrode **44** is put in electrically conducting solution **36** contained in sensor reservoir **34**, and a reference electrode **42** is put in the solution **40** contained in outlet reservoir **38**. Between electrodes **44** and **42**, two resistors **48** and **50** are used to sample the potentials between electrodes **44** and **42** to the data acquisition system **52**. For sample separation, the sample can be injected by hydrodynamic injection or electrokinetic injection methods into capillary **31**, and a high voltage applied between the ground and power electrodes. Sample detection is achieved by sensing the potential difference between the reference electrode **42** and the sensing electrode **44** over time. These techniques are described by S.F.Y. Li in *Capillary electrophoresis: Principles, Practice and Applications*, Elsevier Science Publications, 1992.

The embodiment shown in the above figures can be used for conductivity detection in many methods of electrophoresis. For simplicity, capillary zone electrophoresis (CZE) is chosen for explaining the principle of the present invention. Figures 3A and B show a theoretical electric field across the corresponding section of capillary tube **32**. Figure 3A shows buffer **33** alone. Figure 3B shows buffer **33** with samples **X** and **Y** being separated by CZE. When a high voltage is applied, a straight baseline of electric field across the whole capillary **32** as shown in Fig. 3A is theoretically obtained because the running buffer is homogeneous during CZE. However, some

difference in the l ctric field will exist if a sample is injected into the capillary. If the sample component's mobility, for example **X**, is larger than that of the running buffer, the electric field in the plug of the sample component will be lower than that of the running buffer as shown in Fig. 3B. Conversely, if the sample component's mobility, for example **Y**, is smaller than that of the running buffer, the electric field in the plug of the sample component will be larger than that of the running buffer (Fig. 3B). When the sample components are passing by the region between the fracture **58** and the outlet of the capillary, the potential between electrodes **44** and **42** will change and the analytes A or B can be detected.

A similar design can be used for microchip CE as shown in Fig. 4 and Fig. 5. In this embodiment, only one capillary channel is shown for ease of illustration. It is understood that a CE chip may have numerous channels with various designs. The microchip CE in this example is made of two glass plates **60** and **64**. On bottom glass plate **60** is fabricated separation channel **78**, injection channel **80** connected to sample reservoir **82** and **62**, and sensor channel **68** connected to sensor reservoir **66** and **70**. Sample loading electrode **86**, waste electrode **90**, power electrode **88**, sensing electrode **72** and ground/reference electrode **74** are fabricated to connect to sample reservoir **82**, waste reservoir **62**, inlet reservoir **84**, sensor reservoir **70**, and outlet reservoir **76** respectively. On the top glass plate, access holes (not shown) are drilled to access the corresponding reservoirs and channels on the glass plate **60**. The two glass plates are bonded together during fabrication. The thickness **L1** of conductive wall **7 1** between the detection channel **68** and the separation channel **78** is less

than 40μm, preferably less than 30μm for borate silicate glass. Samples are loaded using the loading and waste electrodes according to standard methods. It has been shown that a thin layer of glass is ion conductive. Based on the same principle described above for CE, sample components in microchip CE can be detected by measuring the potential between the electrodes **72** and **74** during electrophoresis. The distance L2 from the detection channel to the outlet of the separation channel **78** is near or less than the length of the sample plug. For a channel made of glass, this thickness is preferably several tens of micrometers. For microchips made from other types of glass or from other material, the thickness of the conductive wall may be determined by one of ordinary skill in the art without undue experimentation.

Experiments have been done in the laboratory to test the feasibility of the present invention. To separate and detect K^+ and Na^+, 50mM triethanolamine (pH 6.5, adjusted by adding HCl) was used as running buffer for CE. Platinum electrodes were used for applying high voltages. The sensing electrodes were Ag/AgCl wire (diameter, 1 mm) electrodes. Gigaohm (GΩ) resistors were chosen for the resistors **48** and **50**. Data acquisition was obtained through a microprocessor. Fig. 7 and 8 show typical electropherogram obtained. We can see that K^+ and Na^+ ions can be well separated and detected using the present invention.

From the above explanation, we can expect that noise will exist if high voltage is used for electrophoresis, and the voltage is not stable during electrophoresis, as can be seen in the baselines in Figures 7 and 8. To

improv signal /noise ratio (S/N), the ratio of the potential measured to the current generated during CE can be measured using a noise reducing circuit **94**. One embodiment is shown in Fig. 6. The voltage S1 collected from the sensing electrodes and the voltage S2 due to the current I are amplified by A2 and A1. Then the signal S is obtained by dividing the output S1' from A2 by the output S2' from A1 through a divider A3. From figure 6 it can be shown that:

$$I = V/R_o \quad \text{.....(1)}$$

$$S_1 = I \times R_S \times (R_2/(R_1+R_2)) \quad \text{.....(2)}$$

$$S_2 = I \times R_3 \quad \text{.....(3)}$$

$$S_1' = S_1 \times (1+R_4/R_5) \quad \text{.....(4)}$$

$$S_2' = S_2 \times (1+R_7/R_6) \quad \text{.....(5)}$$

$$S = k_1(S_1'/S_2') \quad \text{.....(6)}$$

From Eq. 1-6 :

$$S = k_1(S_1'/S_2') = kR_S \quad \text{.....(7)}$$

Where $k = k_1 \times \{(1+R_4/R5) \times R_2\}/\{R_3 \times (1+R_7/R_6) \times (R_1+R_2)\}$=constant

From the above results, one can see the signal S is proportional to R_S only and not affected by voltage, current or the resistance of the circuit. In other words, this improved circuit can remove the effects of ripple of the

high voltage power supply. Therefore, the baseline noise can be reduced and the ratio of signal to noise will improve.

Those skilled in the art will know that many variations of design can be realized based on the same principle as described above. Although a separate noise reduction circuit **94** is shown in Figure 6, it should be understood by one skilled in the art that other equivalent interfaces are possible. For instance, the noise reducing function of circuit **94** can be incorporated into a sophisticated data acquisition system **52** as part of its internal submodules.

As mentioned above, the distance between the two points where potential difference is measured (e.g. L and L2 in Fig. 2B and 5 respectively) is preferably smaller than the length of the sample plug injected in order to obtain maximum resolution. For capillary electrophoresis, the length of the sample plug injected is typically around 1mm. Therefore, L and L2 are preferably less than 1mm in order to achieve high resolution and sensitivity. Thus good electrical insulation would have to be provided between the two measuring points. Alternatively, a channel with a smaller diameter than that of the separation channel may be provided between the two measuring points such that the distance therebetween may be lengthened without compromising resolution and sensitivity. One example is shown in Fig. 9. In this example, the inlet 101 and outlet ends 107 of a capillary tube 102 is shown. The tube 102 is separated into two parts. Section S1, used for separation, has a larger diameter D1, while section S2, proximate the outlet end in this example, has a smaller inner diameter of D2. A sample 109 of length L3 is shown to

migrate from the inlet to the outlet end. As the sample moves towards section S2, the length of the sample would be lengthened due to the smaller diameter of the channel. If the two measuring points for potential gradient detection (which are fracture 103 and the outlet end in this example) is provided at section S2, it is clear that the distance between these two measuring points may also be proportionately lengthened.

Capillaries with varying diameters can be made by normal commercial machines for making capillaries or pulling one end of a capillary tube with uniform diameter to produce one end with a small diameter after heating the tube. Commercially available machines include Laser-based micropipette pullers, for example the P2000 from Sutter Instrument Co. Channels on microchips having varying sizes can be easily produced through different mask design and performing the appropriate photolithography known in the art. The electrically conductive medium contained within the various sensing, outlet and inlet reservoirs may be the same or different, depending on the applications.

Although fused silica and glass substrates are commonly used as separation channels in CE and microchip CE, other substrates, such as poly(dimethylsiloxane) (PDMS) and PMMA, can be used also.

The present invention can be applied to existing electrophoretic channels by providing conductive elements on them, for example, by bonding some filters on them. The bonding method could be, for example, thermal bonding for many plastics, oxygen plasma bonding for PDMS. For a fused silica capillary, well-known techniques such as fracturing, making a frit (US Reissued patent 035102) and applying polymers after fracturing

(US patent 5169510) may all be applied. For glass chann ls, a thin wall of 1-40μm, preferably 1-20μm, may be used. The most effective thickness is dependent on the quality of the glass, and may be determined by one of ordinary skill in the art by routine experimentation.

The detection channel on microchip CE could be on the top or the bottom of the separation channel rather than lying adjacent to the separation channel. The electrodes for sensing can be other electrodes, such as calomel electrode, platinum and gold. The reference electrode in the outlet reservoir can be combined with the ground electrode. For microchip CE, both sensing electrodes and the electrophoresis electrodes can be microfabricated on the chips or just inserted directly in the buffer reservoirs. It is also possible to create two or more conductive elements on the capillary or the separation channel in order to detect sample components at different places. For example, by having two factures along two different points of a capillary tube. The reference electrode may also be positioned away from the outlet end by creating an additional conductive element and the corresponding reservoir for connection to the reference electrode.

CLAIMS

1. An electrophoretic apparatus comprising:

a power supply with a ground electrode and a power electrode;

a separation channel with an inlet end and an outlet end and containing separation medium, said inlet end electrically coupled to the power electrode of said power supply, said outlet end electrically coupled to the ground electrode of said power supply;

a data acquisition system with a reference electrode and an electrical potential sensing electrode, said reference electrode electrically coupled to said ground electrode; and

a conductive element, provided on said separation channel between said inlet end and said outlet end, said conductive element permitting electrical signals to pass through without detectable bulk flow of separation medium and sample, said sensing electrode electrically coupled to said conductive element

whereby the electrical potential within the separation channel between the conductive element and the outlet end may be detected by said data acquisition system without causing disturbance to the flow of samples in said separation channel.

2. An electrophoretic apparatus comprising :

a capillary electrophoresis array chip containing

a plurality of longitudinally aligned capillary separation channels containing separation medium, each said channel having an inlet end and an outlet end,

a conductive element provided on each of said separation channel proximate the corresponding outlet end, said conductive element allowing electrical signals to pass through without detectable bulk flow of the separation medium and sample;

a plurality of sensor reservoirs containing electrically conductive medium each connected to one of said conductive element of said separation channels;

a power supply with

a ground electrode coupled to each of said outlet ends; and

a power electrode coupled to each of said inlet end

for electrophoresis of samples provided within said channel; and

a data acquisition system with

at least one reference electrode coupled to each of said outlet ends; and

a plurality of electrical potential sensing electrode each coupled to one sensor reservoir

whereby electrical potential within said separation channel between said element and said outlet end is measured by said data acquisition system.

3. A method for detecting samples in an electrophoretic system, said system containing an electrophoretic channel with an inlet end and an outlet end, said channel containing electrophoretic medium and samples, said apparatus further containing an electrically conductive element on the wall of said electrophoretic channel proximate said outlet end, said conductive element permitting electrical signal to pass through without detectable bulk flow of electrophoretic medium and sample, said method comprising :

separating the samples in said electrophoretic channel containing electrophoretic medium by producing an electrical field between the inlet end to the outlet end;

sensing the electrical potential within the channel between the conductive element and the outlet end.

4. An electrophoretic apparatus according to claim 1 further comprising an inlet reservoir connected to said inlet end, said inlet reservoir for retaining electrically conductive medium to which said power electrode is coupled.

5. An electrophoretic apparatus according to claim 1 further comprising an outlet reservoir connected to said outlet end, said outlet reservoir for retaining electrically conductive medium to which said ground electrode is coupled.

6. An electrophoretic apparatus according to claim 1 wherein said conductive element is connected to a sensing reservoir such that said conductive element is electrically connected to said sensing electrode

via electrically conductive medium retained within said sensing reservoir.

7. An electrophoretic apparatus according to claim 1 further comprising

an inlet reservoir connected to said inlet end, said inlet reservoir for retaining said separation medium to which said power electrode is coupled;

an outlet reservoir connected to said outlet end, said outlet reservoir for retaining said separation medium to which said ground electrode is coupled; and

a sensing reservoir connected to said conductive element, said sensing reservoir for retaining an electrically conductive medium to which said sensing electrode is coupled.

8. An electrophoretic apparatus according to claim 1 wherein said separation channel is a capillary tube, and said conductive element is a fracture in said capillary tube.

9. An electrophoretic apparatus according to claim 1 wherein said conductive element is 0.1 to 5mm from the outlet end.

10. An electrophoretic apparatus according to claim 1 wherein said separation channel is a capillary tube, said conductive element is a fracture in said capillary tube 0.1 to 5mm from the outlet end.

11. An electrophoretic apparatus according to claim 1 wherein a second conductive element connected to a second sensor reservoir with electrically conducting medium is provided between said conductive

element and said inlet end, and said reference electrode is electrically connected to said second sensor reservoir, such that electrical potential within the separation channel between said first and second conductive element may be detected by said data acquisition system without causing disturbance to the flow of separation medium and samples in said separation channel.

12. An electrophoretic apparatus according to claim 1 further comprising a first resistor coupled between said reference electrode and said data acquisition system, and a second resistor coupled between said sensing electrode and said data acquisition system for sampling the potential between the reference and sensing electrodes.

13. An electrophoretic apparatus according to claim 12 further comprising a noise reducing means interposed between said sensing electrode and said data acquisition system for removing the ripple effects of said power supply, said noise reducing means comprising :

at least a first amplifier connected to the sensing electrode for amplifying the potential difference between the sensing and the reference electrodes.

at least a second amplifying connected to the ground electrode for amplifying current signals therefrom; and

at least one signal divider having its input coupled to said first amplifier and said second amplifier; the output of said signal divider coupled to said data acquisition system for removing noise from said potential and current signals.

14. An electrophoretic apparatus according to claim 2 further comprising a sample channel connected to each of said separation channel for receiving and loading a sample, and a sample electrode coupled to said sample and said power supply for sample injection.

15. An electrophoretic apparatus according to claim 2 further comprising an inlet reservoir connected to each of said inlet end, said inlet reservoir for retaining separation medium to which said power electrode is coupled.

16. An electrophoretic apparatus according to claim 2 further comprising an outlet reservoir connected to each of said outlet end, said outlet reservoir for retaining separation medium to which said ground electrode is coupled.

17. An electrophoretic apparatus according to claim 2 wherein said conductive element is connected to a sensing reservoir such that said conductive element is electrically connected to said sensing electrode via electrically conductive medium retained within said sensing reservoir.

18. An electrophoretic apparatus according to claim 2 further comprising an inlet reservoir connected to each of said inlet end, said inlet reservoir for retaining separation medium to which said power electrode is coupled;

an outlet reservoir connected to each of said outlet end, said outlet reservoir for retaining said separation medium to which said ground electrode is coupled; and

a sensing reservoir connected to said conductive element, said sensing reservoir for retaining an electrically conductive medium to which said sensing electrode is coupled.

19. An electrophoretic apparatus according to claim 16 wherein said outlet reservoirs are interconnected.

20. An electrophoretic apparatus according to claim 18 wherein said outlet reservoirs are interconnected.

21. An electrophoretic apparatus according to claim 15 wherein said inlet reservoirs are interconnected.

22. An electrophoretic apparatus according to claim 18 wherein said inlet reservoirs are interconnected.

23. An electrophoretic apparatus according to claim 2 wherein said electrophoretic chip is made of glass, and said conductive element is a thin wall separating said channel and said sensor reservoir, said thin wall of less than 40μm thick.

24. An electrophoretic apparatus according to claim 2 wherein said conductive element is less than 5mm from the outlet end.

25. An electrophoretic apparatus according to claim 2 wherein said conductive element is a thin wall separating said channel and said

sensor reservoir, and the length of said conductive wall along th longitudinal axis of said channel is less than 10mm .

26. An electrophoretic apparatus according to claim 2 wherein a second conductive element connected to a second sensor reservoir with electrically conducting medium is provided on said channel between said conductive wall and said inlet end, and said reference electrode is electrically connected to said second sensor reservoir, such that electrical potential within the separation channel between said first and second conductive elements may be detected by said data acquisition system without causing disturbance to the flow of separation medium and samples in said separation channel.

27. An electrophoretic apparatus according to claim 2 further comprising

a sample channel connected to said separation channel for receiving and loading a sample;

a sample electrode in electrical contact with said sample in said sample channel and coupled to said power supply for sample injection;

a waste channel connected to said sample reservoir for receiving and retaining excess samples; and

a waste electrode in electrical contact with said sample in said waste channel and coupled to said power supply for controlling the sample injection process.

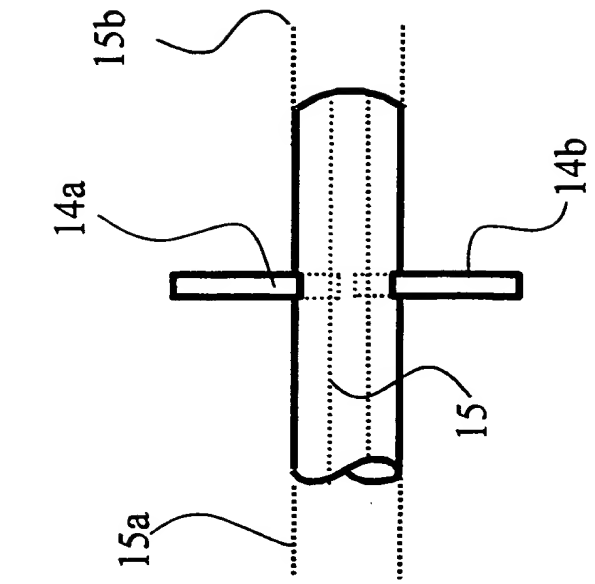

Figure 1B

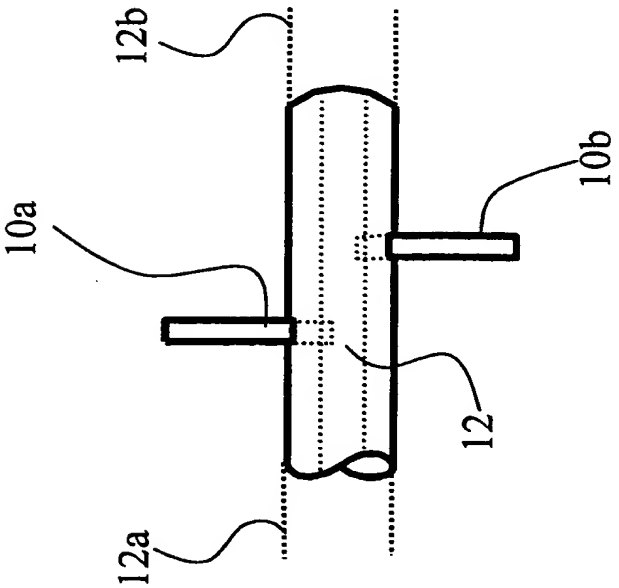

Figure 1A

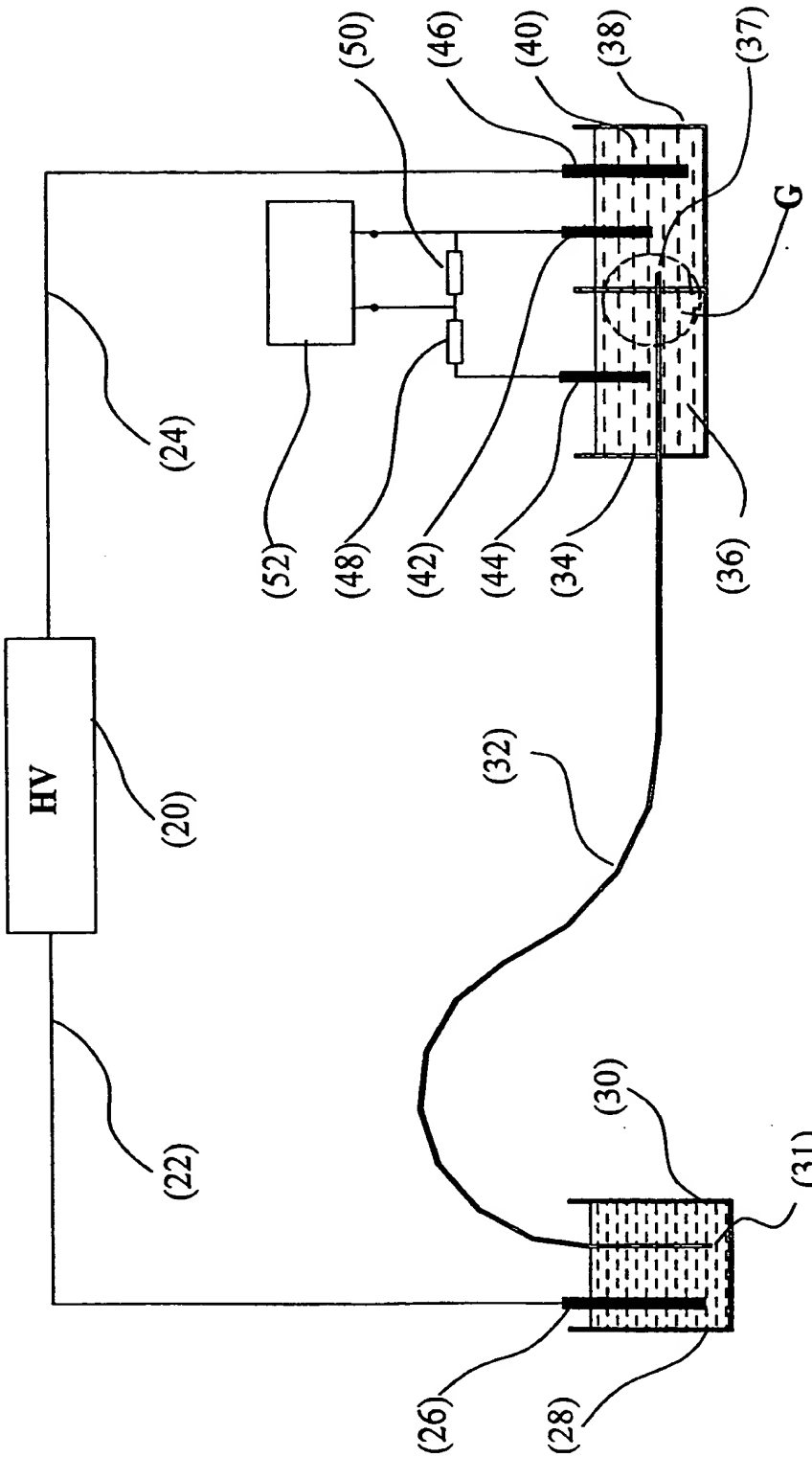

Figure 2A

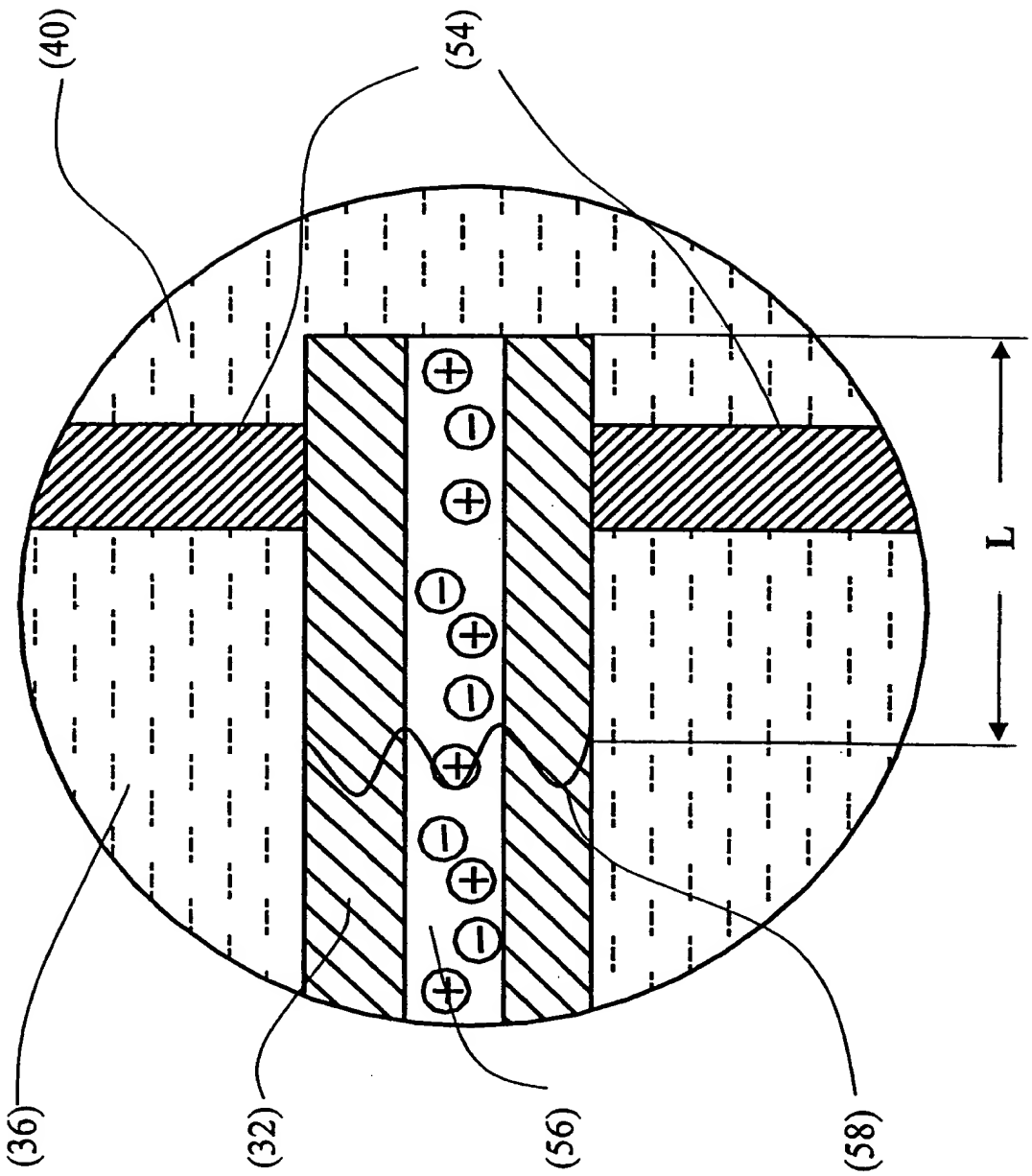

Figure 2B

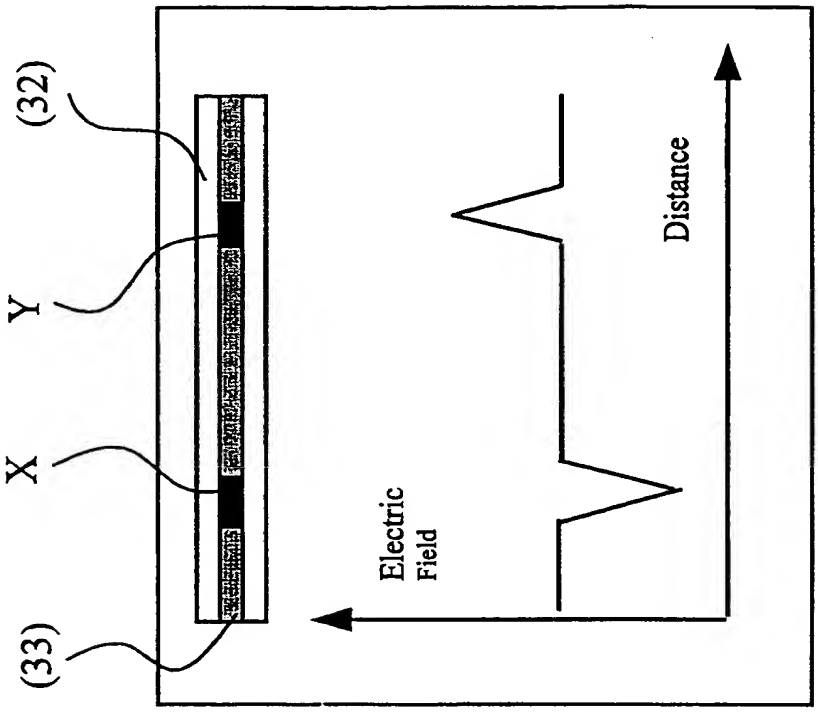

Figure 3B

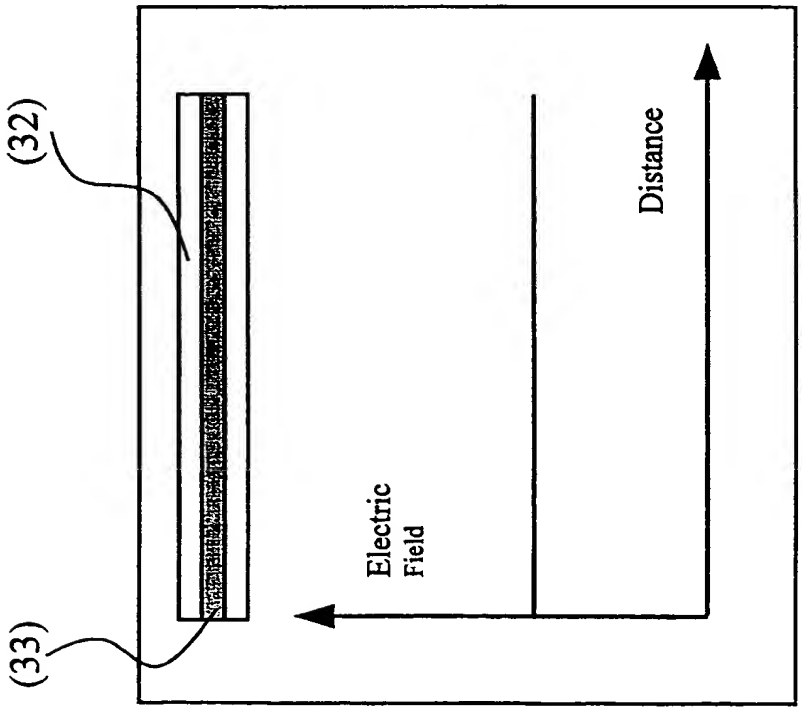

Figure 3A

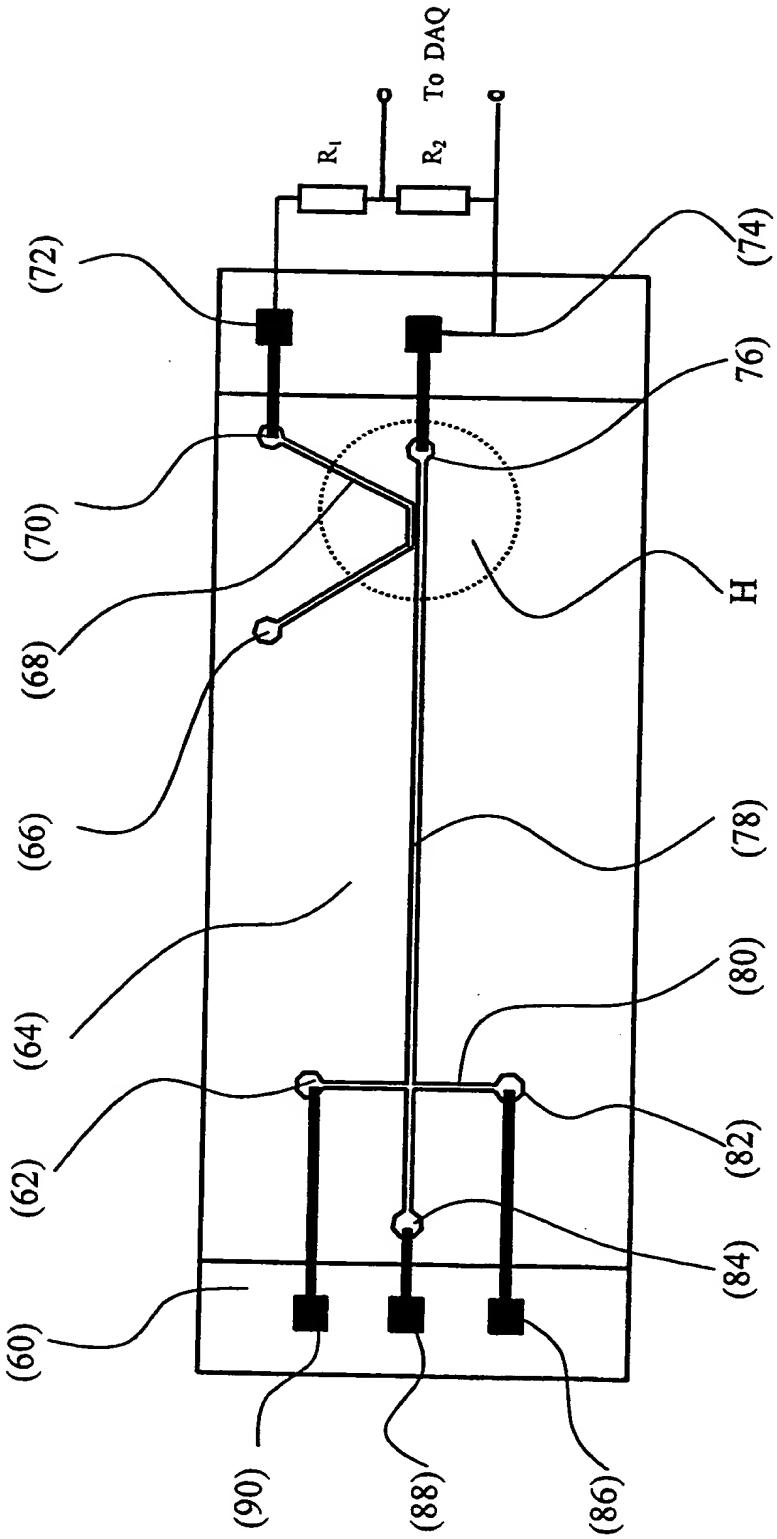

Figure 4

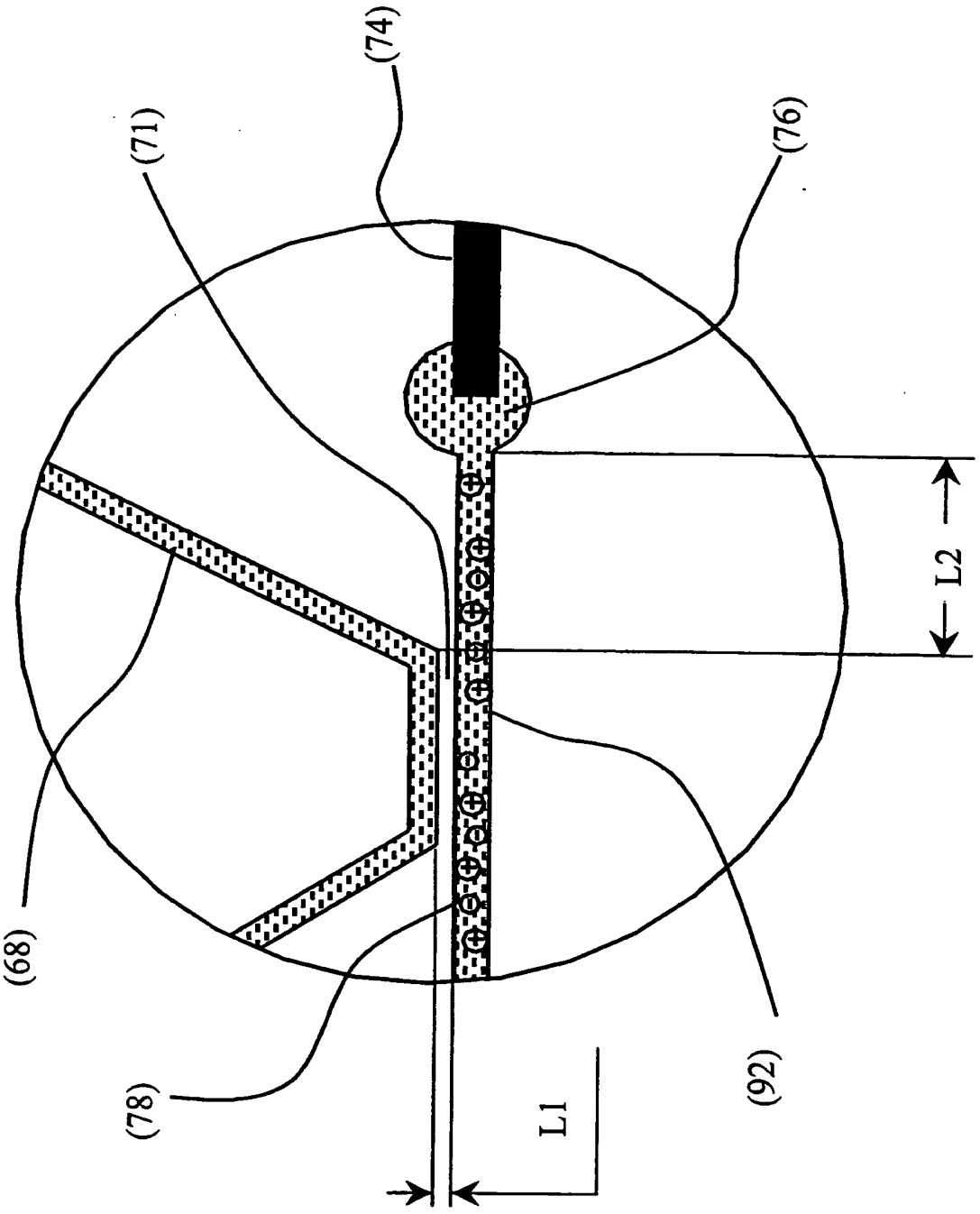

Figure 5

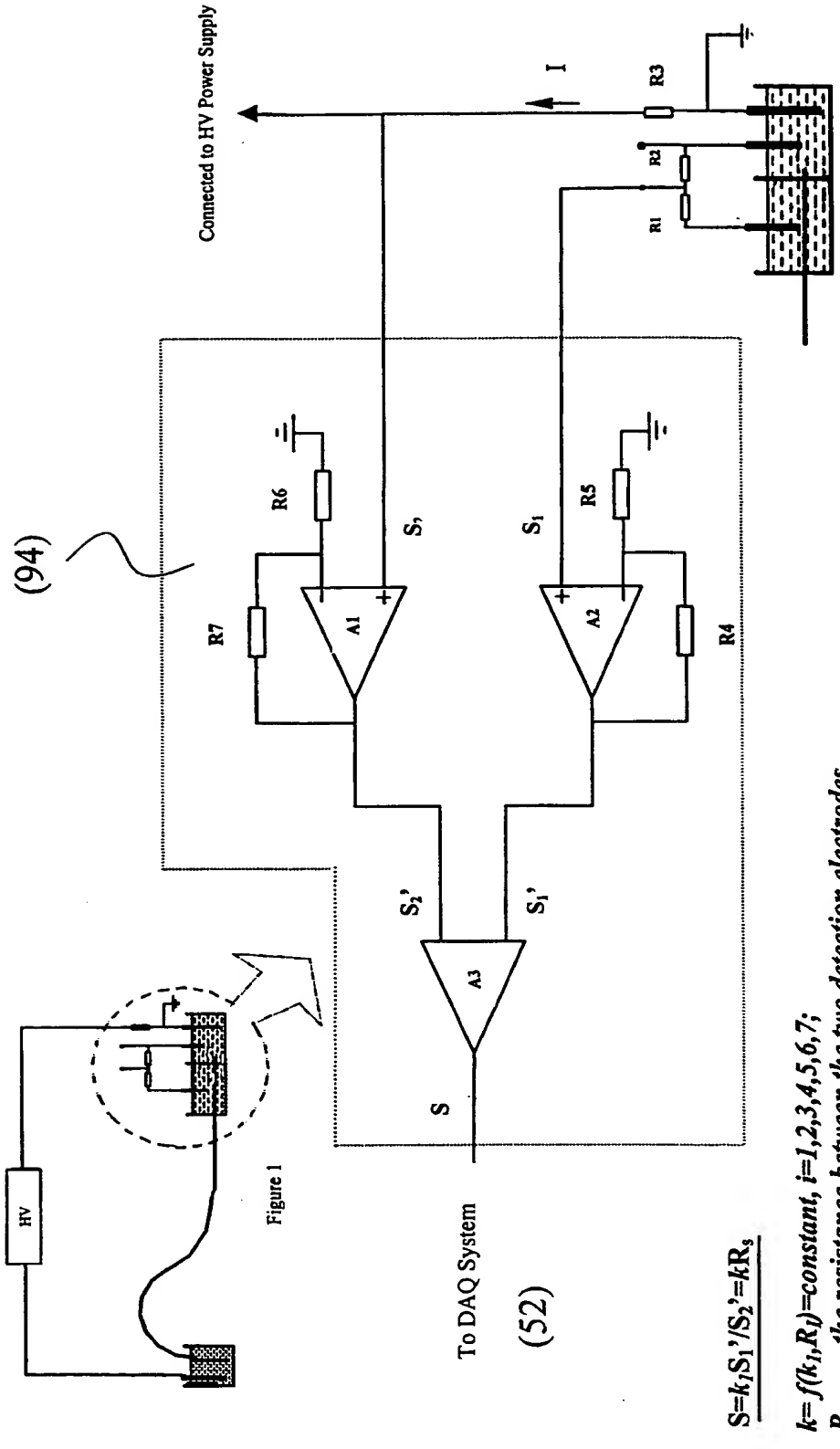
Connected to HV Power Supply
I
R3
R2
R1
(94)
R6
R5
S_7
S_1
R7
A1
A2
R4
S_2'
S_1'
A3
S
To DAQ System
(52)
HV
Figure 1
$S=k_i S_1'/S_2'=kR_s$
$k=f(k_i,R_i)$=constant, i=1,2,3,4,5,6,7;
R_s—— the resistance between the two detection electrodes.

Figure 6

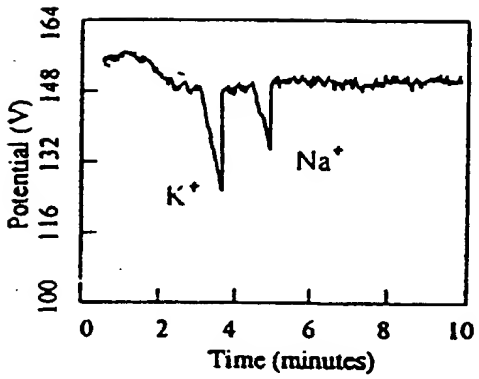

FIG.7

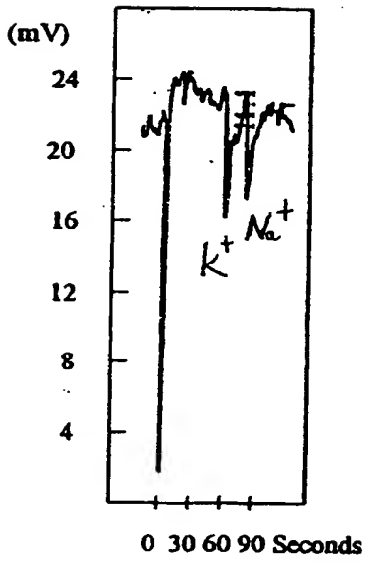

FIG.8

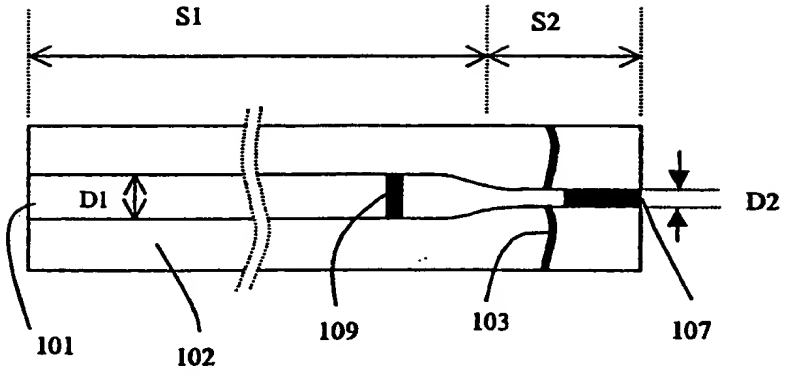

FIG.9

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SG 00/00077

CLASSIFICATION OF SUBJECT MATTER

IPC[7]: G01N 27/447

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC[7]: G01N 27/447

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JP 11-108890 A (YOKOGAWA ELECTRIC CORP.) 23 April 1999 (23.04.99) (abstract), In: Patent Abstracts of Japan [CD-ROM].	1,2,3

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

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„O" document referring to an oral disclosure, use, exhibition or other means
„P" document published prior to the international filing date but later than the priority date claimed

„T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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„Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
„&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 July 2000 (26.07.2000)	9 October 2000 (09.10.2000)
Name and mailing adress of the ISA/AT Austrian Patent Office Kohlmarkt 8-10; A-1014 Vienna Facsimile No. 1/53424/535	Authorized officer Nardai Telephone No. 1/53424/347

Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/SG 00/00077

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
JP A2 11108890	23-04-1999	none	